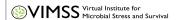
Mapping the two-component regulatory networks in *Desulfovibrio vulgaris*

DOE GENOMICS:GTL

Lara Rajeev, Eric Luning, Paramvir Dehal, Marcin Joachimiak, Adam Arkin, Aindrila Mukhopadhyay Physical Biosciences Division, Lawrence Berkeley National Lab

Ecosystems and Networks Integrated with Genes and Molecular Assemblies







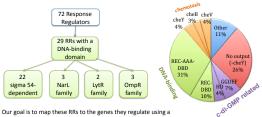


http://vimss.lbl.gov/

Introduction

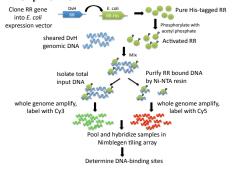
D. vulgaris Hildenborough has 72 response regulators

The Desulfovibrio are sulfate reducing bacteria that are important in the sulfur and carbon cycles in anoxic habitats. Its large number of two component systems are probably critical to its ability to sense and respond to its environment.



Our goal is to map these RRs to the genes they regulate using a DNA-affinity-purification-chip (DAP-chip) protocol

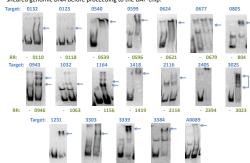
DAP-Chip Workflow



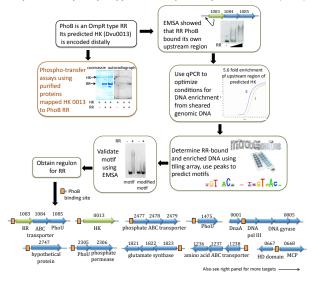
Results

First target determined using EMSA

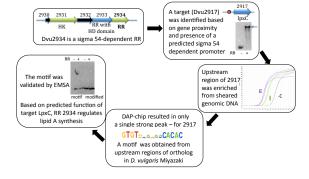
A positive target was determined for as many RRs as possible using EMSA. Targets were selected based on gene proximity, regulon predictions and/or predicted sigma54 dependent promoters. qPCR was used to ensure that the target was enriched from sheared genomic DNA before proceeding to the DAP-chip.



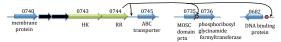
Example of a completely mapped two component system: Dvu1083 (PhoB)



Example of an RR with only one target: Dvu2934

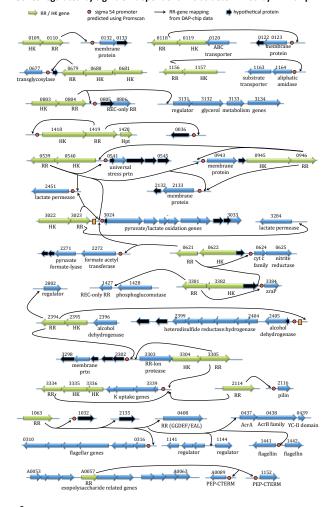


Example of a DAP-chip done blind: Dvu0744



RR 0744 did not bind its own upstream region, and no predicted sigma 54 promoter was found in any of the proximal genes. A DAP-chip was done directly, and the top three targets are shown. Motif predictions and validations are ongoing.

Genes regulated by sigma 54-dependent RRs as determined by DAP-chip



DAP-chips were done for 20 RRs. Two RRs Dvu1083 and Dvu2934 have been completely mapped. Motif predictions and validations are ongoing for the rest, DAP-chips for most of the remaining 9 RRs will be done blind since we could not identify a target for them.

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